

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Page 2

In the claims

1. (Presently Amended) A method for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample comprising:
 - a) co-encapsulating in a micro-environment selected from a liposome, bead, cell, ghost red blood cell and ghost macrophage an environmental library comprising a mixture of target DNA obtained from more than one a mixed population of organisms with a mixture of DNA probes comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified activity;
 - b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences in the co-encapsulated mixture; and
 - c) screening the micro-environment to recover the hybridized complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified activity in the recovered target DNA.
2. (Previously Amended) The method of claim 1, further comprising transforming host cells with the recovered target DNA to produce an expression library of a plurality of clones.
3. (Presently Amended) The method of claim 1, wherein the [more than one] mixed population of organisms is a plurality of microorganisms.
4. (Original) The method of claim 3, wherein the microorganisms are uncultured microorganisms.

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Page 3

5. (Previously Amended) The method of claim 2, further comprising screening the expression library for the specified enzyme activity.

6. (Previously Amended) The method of claim 1, wherein the screening to recover the hybridized complementary sequences comprises:

- a) converting double stranded DNA into single stranded DNA;
- b) recovering from the converted single stranded DNA, single stranded target DNA which hybridizes to probe DNA;
- c) converting recovered single stranded target DNA to double stranded DNA; and
- d) transforming a host cell with the double stranded DNA of c).

Claim 7 (cancelled)

8. (Amended) The method of claim 1, wherein said target DNA is gene cluster DNA.

9. (Previously Amended) The method of claim 4, wherein the uncultured microorganisms are obtained from an environmental sample.

10. (Original) The method of claim 4, wherein the uncultured microorganisms comprise a mixture of terrestrial microorganisms or marine microorganisms or airborne microorganisms, or a mixture of terrestrial microorganisms, marine microorganisms and airborne microorganisms

11. (Original) The method of claim 2, wherein the clones comprise a construct selected from the group consisting of phage, plasmids, phagemids, cosmids, fosmids, viral vectors, and artificial chromosomes.

12. (Original) The method of claim 1, wherein the target DNA comprises one or more operons, or portions thereof, of the DNA population.

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Page 4

13. (Original) The method of claim 12, wherein the operon or portions thereof encodes a complete or partial metabolic pathway.

14. (Original) The method of claim 4, wherein the uncultured microorganisms comprise extremophiles.

15. (Original) The method of claim 14, wherein the extremophiles are selected from the group consisting of thermophiles, hyperthermophiles, psychrophiles, barophiles, and psychrotrophs.

16. (Original) The method of claim 6, wherein the host cell is selected from the group consisting of a bacterium, fungus, plant cell, insect cell and animal cell.

17. (Original) The method of claim 1, wherein the target DNA encodes a protein.

18. (Original) The method of claim 17, wherein the protein is an enzyme.

19. (Original) The method of claim 18, wherein the enzyme is selected from the group consisting of oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.

Claim 20 (cancelled)

21. (Presently amended) The method of claim 20 1, wherein the liposomes are prepared from one or more phospholipids, glycolipids, steroids, alkyl phosphates or fatty acid esters.

22. (Original) The method of claim 21, wherein the phospholipids are selected from the group consisting of lecithin, sphingomyelin and dipalmitoyl.

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Page 5

23. (Presently amended) The method of claim 20 21, wherein the steroids are selected from the group consisting of cholesterol, cholestanol and lanosterol.

24. (Presently amended) The method of claim 1, wherein the detectable ~~marker is label~~ comprises a fluorescent dye, a visible dye, a bioluminescent material, a chemiluminescent material, a radioactive material, or an enzymatic substrate.

25. (Original) The method of claim 24, wherein the bioluminescent material is green fluorescent protein (GFP) or red fluorescent protein (RFP).

26. (Presently amended) The method of claim 25 24, wherein detection of the fluorescent dye or ~~a~~ the visible dye is carried out by fluorometric or spectrophotometric measurement.

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Page 6

27. (Presently amended) A method for enriching an environmental library for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample comprising:

- a) co-encapsulating in a micro-environment selected from a liposome, cell, ghost red blood cell and ghost macrophage a an environmental library comprising a mixture of target DNA obtained from ~~more than one~~ a mixed population of organisms with at least one DNA probe comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity;
- b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences in the mixture; and
- c) screening to recover the hybridized complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified activity in the recovered target DNA.

28. (Presently amended) A method for enriching an environmental library for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample comprising:

- a) co-encapsulating in a micro-environment selected from a liposome, cell, ghost red blood cell and ghost macrophage a target DNA obtained from ~~more than one~~ a mixed population of organisms with a mixture of DNA probes comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity;
- b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences in the mixture; and

PATENT
ATTORNEY DOCKET NO.: DIVER1280-11

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Page 7

c) screening to recover the hybridized complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified activity in the recovered target DNA in the environmental library.